STUDIES ON CHORIOMENINGITIS AND POLIOMYELITIS

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CHARLES ARMSTRONG

Senior Surgeon, U. S. Public Health Service*

CENTRAL nervous system ailments known to be or suspected of being caused by viruses are apparently on the increase and they constitute a puzzling diagnostic problem for the clinician.

For instance, a group of cases characterized by fever with symptoms of meningeal irritation and a clear to slightly turbid, sterile-to-culture spinal fluid showing a lymphocytic cellular response has been considered as a clinical entity under such varied designations as lymphocytic or aseptic or idiopathic meningitis. Laboratory investigators have now split certain etiological entities such as lymphocytic choriomeningitis and pseudolymphocytic choriomeningitis from this group of cases, but there still remains a residue of cases of unknown etiology.

These developments denote progress, yet I fear that the development of an etiological classification has rendered the clinician's problem more complex, since, in the individual case of central nervous infection, an etiological diagnosis is usually not possible on clinical findings alone, but must rest upon laboratory determinations. It is by this fact that I would justify this essentially laboratory presentation of certain aspects of lymphocytic choriomeningitis and of poliomyelitis.

LYMPHOCYTIC CHORIOMENINGITIS

The virus of lymphocytic choriomeningitis was first isolated and described in 1934 at the National Institute of Health.[1] The virus was first isolated with certainty from a human case by Scott and Rivers in 1935 and has subsequently been isolated from widely separated portions of the United States[1, 2, 3, 4, 5, 6, 7, 8] and in England,[9, 10] France[11, 12] and

* From the Division of Infectious Diseases, National Institute of Health, Washington, D. C.

Japan[13] and in northern Africa,[14] and there is clinical and serological evidence of its presence in Ireland.[15] There are, moreover, reasons for feeling that the virus is probably world-wide in distribution.

The symptoms as described for etiologically proven cases of choriomeningitis have varied markedly although the viruses isolated therefrom have been immunologically similar with the exception that MacCallum, Findlay and Scott[16] studied two cases which clinically resembled choriomeningitis from which identical strains of virus were isolated. These viruses simulated choriomeningitis in both their clinical and pathological manifestations in experimentally inoculated monkeys and mice, but were found to be immunologically distinct from choriomeningitis and were designated as the virus of pseudochoriomeningitis.

Symptomatology: A sufficiently large number of proven cases of choriomeningitis virus infection have not as yet been observed to assure that all the clinical manifestations of the disease have as yet been identified. However, several clinical types of the disease are now known to exist.

1. *A grippal or non-nervous system type:* Many persons who deny having had any central nervous system ailment whatever have been found to harbor specific virus-neutralizing antibodies in their serums, which suggests the occurrence of a systemic type of infection without central nervous system involvement. This assumption is supported by the fact that susceptible animals, when inoculated with the virus by routes other than directly into the central nervous system, usually develop symptoms without evidence of meningeal or brain involvement; and French investigators[17] have shown the same to be true for human volunteers inoculated subcutaneously with the virus. That such human cases also occur in nature has recently been demonstrated at the National Institute of Health[18] where the first spontaneously acquired case of this systemic type yet reported[18] was observed. The case occurred in a 31 year old man engaged in choriomeningitis research. On March 11, 1940, he developed moderate fever with troublesome pains in his arms and back (Fig. 1). During the next three days his fever increased to 38.8° C. but anorexia, malaise, marked prostration, and lumbar pains so severe as to require codeine were his only complaints. His face was somewhat flushed but otherwise the physical examination was negative. There was no headache, or stiffness of the neck or altered reflexes. A blood count on the fourth day of illness revealed 2900

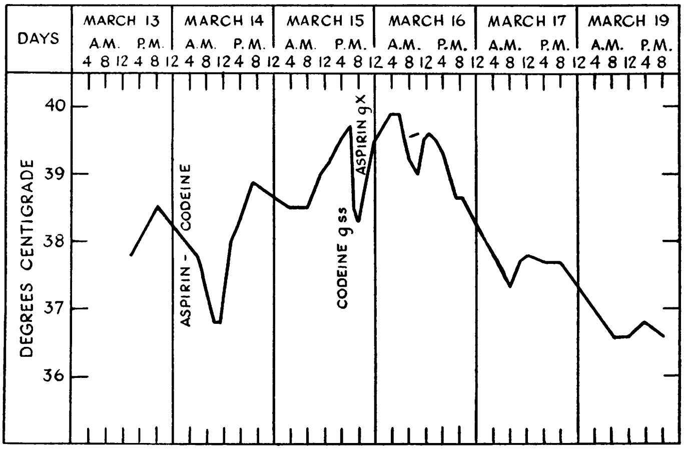

Fig. 1—Temperature course in patient with systemic choriomeningitis virus infection without central nervous system involvement.

white cells of which 45 per cent were polymorphonuclear neutrophiles, 50 per cent lymphocytes, and 5 per cent mononuclear cells. On the fifth day of illness his temperature reached its height, 39.9° C. and there was marked prostration and backache. The next day the fever was lower and his temperature reached normal on the seventh day accompanied by marked amelioration of the pains. Weakness and prostration were marked, however, and persisted for a full week following the return of his temperature to normal. The clinician in charge considered the case to be one of uncomplicated influenza.

There was no indication for a spinal tap and none was done. Blood drawn on the fifth day of illness and inoculated into white mice induced the typical clinical and pathological picture of choriomeningitis and the virus was immunologically identified. Blood drawn in November, 1939 was negative when tested for specific antibodies, while a sample drawn on April 28, 1940 was markedly protective.

This identification of an influenza-like ailment due to the virus of choriomeningitis is of interest in view of the fact that approximately 11 per cent of 2000 sera collected at random from various parts of the United States contained antibodies for this virus, but with rare exceptions the donors when interrogated denied any history of central nervous affection. It is possible, therefore, that a portion of the cases of

"grippe" or "influenza," especially in interepidemic periods may be due to the virus of choriomeningitis.

2. *Meningeal type:* The central nervous system symptoms are often preceded by upper respiratory or influenza-like manifestations which usually improve, to be followed in several days by the sudden appearance of fever with meningeal symptoms such as severe headache, vomiting, stiff neck, and by positive Kernig and Brudzinski signs. The spinal fluid is clear, sterile-to-culture and may contain as many as 3000 cells per cmm., mainly lymphocytes.

3. *Meningo-encephalomyelitic type:* In addition to such purely meningeal symptoms, somnolence, disturbances of deep reflexes, paralysis, and anesthesias have been observed in etiologically established cases. These latter symptoms suggest the presence of encephalomyelitis, for which reason Kreis[19] has justly criticized the designation "choriomeningitis" as being too restrictive in certain cases.

Recovery is usually complete although sequelae probably related to disturbances in the cerebrospinal fluid drainage have been noted in certain cases. Barker and Ford's[5] patient developed persistent symptoms which led to a laminectomy being performed at which time the piarachnoid space was found to be obliterated by fibrous tissue. Other cases have shown a tendency for one to several relapses to occur.

Pathology: No pathological report of an etiologically proven case of choriomeningitis has as yet been made. Viets and Warren[20] have recorded the pathological findings in a patient with acute lymphocytic meningitis who died with convulsions on the fourteenth day of illness in 1934. The etiology in this case was not determined; the choroid plexus was not examined, and the pathological changes reported for the brain were at variance with those usually observed in experimentally inoculated monkeys. We, therefore, do not feel justified in presenting this case as one of probable choriomeningitis infection.

The case reported by Machella, Weinberger, and Lippincott in 1939[21] was, however, clinically suggestive of this ailment and the pathological findings were similar to those observed in experimentally inoculated monkeys. In this case the meninges were infiltrated with lymphocytes and macrophages and were markedly thickened due to connective tissue multiplication with obliteration of the subarachnoid space. The brain substance was in the main not affected. The ventricles were distended, denuded of their ependyma, and there was a narrow

subependymal zone where perivascular lymphocytic infiltration, glial proliferation, and engorgement of vessels and hemorrhages were marked. The choroid plexus was partially necrotic and infiltrated with inflammatory exudate.

Pathology in monkeys: In intracerebrally inoculated monkeys, as described by Lillie,[22] the lesions are characterized by an almost constant, irregular, more or less pronounced lymphocytic infiltration of the choroid plexus, sometimes with an exudate into the ventricles, and by an almost constant, moderate, irregularly distributed infiltration of the pia mater, and by few foci of glial cells and lymphocytic infiltration about the vessels. The cellular infiltration may persist for a long time following the infection. The lungs often present congestion with intracellular edema, serous exudation and perivascular lymphocytic infiltration. Focal interstitial and perivascular accumulations of lymphocytes are frequent in various organs. In this connection it is interesting to note that Armstrong, Wooley, and Onstott[23] and Mendoza[24] have shown the virus to be widely distributed in abundance throughout various organs and tissues of infected monkeys, namely, the adrenals, cerebrum, blood, kidneys, liver, lungs, lymph glands, marrow, heart-muscle, voluntary muscle, pancreas, spinal cord, spinal fluid, spleen, submaxillary glands and testicles, and in amounts not explainable by the presence of virus-containing blood in the vessels. The bile contained no demonstrable virus.

Pathology in mice: Following intracerebral inoculation mice show the same type of meningeal infiltration as noted for monkeys, especially marked at the base of the brain; a lymphocytic infiltration of the choroid plexus with a resulting marked thickening being especially noticeable.

When freshly isolated mouse strains are inoculated subcutaneously or intraperitoneally the central nervous system usually escapes involvement; however, the animals develop marked difficulty of respiration and at autopsy the chest cavity is found filled with a clear virus-containing fluid, the spleen is enlarged and the liver and kidneys often show extreme grades of fatty degeneration. Lesions are entirely lacking when the brain-adapted virus is similarly inoculated.

Host range of the virus: Men, chimpanzees, monkeys, guinea pigs, white rats, cotton rats, rice rats, white mice, gray mice, and dogs are susceptible to the virus. Susceptible birds have not, to date, been found,

although chickens, young chicks, canaries, and pigeons have been tried. These negative results in chickens are of interest in view of the successful cultivation of the virus in the tissue of the developing hen's egg by Bengtson and Wooley.[25] The tissues of the inoculated embryo are rich in virus and many embryos die about the ninth to tenth day, although some of the eggs bring forth living chicks. Such chicks appear rather less active and rather somnolent but usually soon become normal. Laigret and Durand[26] reported the finding of the virus in an uninoculated chick embryo, an extremely interesting result if confirmed.

Personally observed cases: During a period of 17 months five* cases of choriomeningitis were identified in or near the District of Columbia,[7, 27, 28] and one at Lancaster, Pennsylvania.[27]

In five of these cases the virus was recovered from the spinal fluid and in one case where spinal fluid was unattainable the identity of the ailment was confirmed by establishing the absence of specific antibodies early in the disease and their presence subsequent to recovery.

In these six cases, as has been the rule with all reported cases, there was no evidence of contact infection.

This absence of any suggestion of communicability of the disease from person to person raises the question as to the source of the infection.

Virus in mice from infected homes: The knowledge that spontaneous infection with choriomeningitis virus had been detected in mice, monkeys, and dogs naturally suggested the existence of an animal reservoir for the virus from which man becomes infected. With a view to testing this hypothesis the homes of these six patients were investigated as to the presence of vermin, mice, rats, and pets.

Gray mice *(Mus musculus musculus)* were trapped in each home and the virus of choriomeningitis was recovered from a pooled emulsion of livers and spleens of mice captured in *five* of the six homes.

The one failure occurred in connection with the case of a taxicab driver who lived in a poor, isolated abode in nearby Maryland. The patient stated that the home had been overrun with mice and that he had trapped twelve of them from the pantry shortly prior to his illness and that he had also set poison. There was abundant evidence of previous mouse infestation and extensive trapping was undertaken, but only two mice were caught and both proved negative for virus. Thus, while ex-

* Exclusive of two laboratory infections observed during the same period.

posure to mice was established for this patient, we were not able to prove that the mice from the home were actually carrying the virus of choriomeningitis.

One home where infected mice were trapped also kept a dog which was examined but no virus was isolated from its liver or spleen.

Virus studies on mice from seventy-eight homes: More than 400 mice were trapped in homes from various parts of Washington, of which 369 mice survived examination. Of this number 307 were etherized and one kidney and a portion of liver and spleen from each mouse was emulsified in buffered saline (pH 7.6) and 0.03 cc. of the emulsion was inoculated intracerebrally into each of four white mice. When illness resulted, the symptoms and time of death were recorded and a representative sample of forty-six brains from ill white mice were submitted to Surgeon R. D. Lillie, who reported the pathological lesions of choriomeningitis as present in forty-four of them. In two instances the lesions of secondary infection were present.

The final diagnosis of choriomeningitis infection was made, however, by the intracerebral inoculation of four normal mice and of four mice which had been previously immunized to our original strain of choriomeningitis virus.

The inoculation dose of virus employed was 0.03 cc. of a 1:500 suspension of the suspected mouse brain, and in every instance where choriomeningitis virus was finally considered to have been recovered from gray mice, the controls died, while two or more of the immune mice survived. Judged by these criteria choriomeningitis virus was recovered from 65 of a total of 307 gray mice, or approximately one out of every five mice examined was a carrier of the virus. The mice examined were from 78 different homes of which 35 harbored infected mice. Thus it appears that 45 per cent of the mouse infested homes studied were harboring mice infected with choriomeningitis virus. From these 35 infected homes a total of 123 mice were examined of which 65, or 52.8 per cent were carriers of active virus.

The method employed in the above-mentioned studies might be criticized in that white mice were employed as an indicator of infection, since stocks of white mice have on several occasions been found to be spontaneously infected with choriomeningitis virus and it is conceivable that our results were due to the presence of the infection in our white mice rather than in the gray mice which we had trapped.

We feel that this criticism is not valid for the following reasons:

1. The same stock of mice were employed in other virus studies but in no instance was choriomeningitis encountered.

2. It was noteworthy that mice trapped from certain homes were repeatedly found infected while from other households they were consistently negative, a situation which scarcely would have prevailed had we been dealing with a random infection of our stock mice.

3. Gray mice in a number of instances were found to present lesions such as a pleural exudate, fatty liver, and enlarged spleen, which enabled us to predict and later to verify the presence of the virus.

Immunity among trapped gray mice: In order to eliminate all criticism of the employment of white mice as an indicator of the presence of virus, a further test was undertaken. This study was aimed at determining the immunity of gray mice to choriomeningitis, a procedure in which white mice were not employed. Sixty-two gray mice were, therefore, trapped from 22 homes where infected mice had been previously found. These mice were inoculated intracerebrally with 10 to 15 M.L.D. of our original strain of choriomeningitis virus. Of these 62 mice, 41 survived while 21 died, indicating immunity in 66 per cent.

As a control to this group, 47 gray mice trapped in abodes where only non-infected mice had been found, were similarly inoculated, of which only 5, or 10.6 per cent, survived, while 12 white mice employed as additional controls, all died.

The 22 homes from which mice harboring choriomeningitis virus had been trapped and which supplied the 62 mice employed in this immunity test, had supplied 83 mice which were tested for virus, of which 37, or 47 per cent, were found to be carriers. The two methods, therefore, give confirmatory results. The somewhat higher incidence of immunity as compared to active infection (66:47) is what might be expected and suggests that a portion of the mice had probably freed themselves of readily detectable virus but retained their immunity.

Significance of choriomeningitis in mice: The six cases of choriomeningitis in man were widely separated, one in Lancaster, Pennsylvania, two in North West Washington, one in South East, and one in and one adjoining North East Washington. There was no history of contact between any of the cases. Five of the six cases were from homes harboring infected gray mice.

Now if we recall that of 78 homes harboring mice there were 35

which harbored choriomeningitis-infected mice and supplied 5 human cases, while 43 homes harboring non-infected mice supplied only 1 human case, it would appear that these findings are statistically significant, especially when we add to the latter group the large but undetermined proportion of homes which harbored no mice at all and which had no recognized cases of choriomeningitis.

Are mice infected from people or people from mice? There is no recorded instance in which one case had contracted choriomeningitis from another, but on the other hand, a number of cases of the disease have developed among laboratory personnel handling infected mice. The wider extent of the infection in mice, as compared to its recognized human prevalence in the District of Columbia, also suggests that mice, not men, are the reservoir for the infection. The patient noted by Findlay, Alcock, and Stern,[9] who developed choriomeningitis shortly after he had cleaned a shed overrun by mice points in the same direction. Moreover, the tendency for protective antibodies to be relatively most prevalent in the lower economic stratum of society, as noted by Wooley and Armstrong, is hard to explain upon a person-to-person concept of spread, but would be the expected, were mice the source of the infection.

Moreover, the monthly distribution of cases (Table I and Fig. 2) is not inconsistent with this conception. For instance, the Fall peak of cases may be due to the closing of the homes with both mice and men seeking warmer quarters therein. The Spring peak on the other hand, may possibly be related to the birth of litters of infected young during the Spring breeding season.

Choriomeningitis virus in mice: Mice are not readily infected with choriomeningitis through feeding of the virus or by exposure to experimentally inoculated cage mates. It would, therefore, appear improbable that five out of the six cases of choriomeningitis should have in every instance infected the mice in their respective homes especially since in each instance the patient was removed to the hospital during the first days of the illness.

On the other hand, Traub,[29] also Haas[30] have shown that the virus passes readily from the infected mother to her *in utero* young, and that such congenitally infected mice survive birth and may carry the virus for months, while mice infected after birth tend to free themselves rapidly of the virus.

Moreover, while it has been shown that experimentally inoculated

TABLE I

CHORIOMENINGITIS CASES

No.	Color	Age	Sex	Onset		Highest Cell Count Sp. Fluid	Identification-Laboratory		Reported by
				Month	Year		Virus	Neut.	
1	W	19	F	May	1931	3200	−	+	Armstrong & Dickens, P.H.R., June 21, 1935, Vol. 50:831-842
2	W	33	M	Oct.	1931	1520	−	+	Armstrong & Dickens, P.H.R., June 21, 1935, Vol. 50-831-842
3	W	28	M	Mar.	1934	1260	−	+	Armstrong & Dickens, P.H.R., June 21, 1935, Vol. 50:831-842
4	W	3½	F	Nov.	1934	2155	−	+	Collis (Ireland) British Med. J. 1935, *11*: 148[15]
5	W	31	M	Dec.	1934	1700	+	+	Scott, McNair & Rivers, J. Exp. Med. 1936, Vol. 63: 397-414[4]
6	W	33	M	Dec.	1934	720	+	+	Scott, McNair & Rivers, J. Exp. Med. 1936, Vol. 63: 397-414[4]
7	W	20	F	Mar.	1935	409	−	+	Armstrong & Dickens, P.H.R., June 21, 1935, Vol. 50:831-842
8	W	46	M	Oct.	1935	138	+	−	Findlay, Alcock & Stern, Lancet, 1936, Vol. 230: 650-655[9]
9	W	36	M	Oct.	1935	63	+	−	Findlay, Alcock & Stern, Lancet, 1936, Vol. 230: 650-655[9]
10	W	14	F	Oct.	1936	−		+	Dominick, J. A.M.A., 1937, Vol. 109: 247-250
11	W	19	F	Oct.	1936	1380	−	+	Dominick, J. A.M.A., 1937, Vol. 109: 247-250
12	W	26	M	Feb.	1937	2800	+	+	Howard, J. Inf. Dis. 1939, Vol. 64: 66-77[6]
13	W	37	F	Feb.	1936	500	+	−	Barker & Ford, J. A.M.A., 1937, 109: 785-786[5]
14	?	9	?	Apr.	1936	60	+	−	Lesne—cited from Kreis
15	W	11	F	Oct.	1938	101	+	−	MacCallum & Findlay, Lancet, 1939, June 17: 1370[10]
16	C	30	F	Sept.	1939	....			Armstrong, Wallace & Ross, P.H.R. 1940, July 5, *55*:1222[27]
17	?	?	F	May	1938	?	+	+	N. Paul Hudson—*Personal communication*[8]

TABLE I—*Continued*

CHORIOMENINGITIS CASES

No.	*Color*	*Age*	*Sex*	*Onset*		*Highest Cell Count Sp. Fluid*	*Identification-Laboratory*		*Reported by*
				Month	*Year*		*Virus*	*Neut.*	
18	W	28	F	Apr.	1938	....	+	−	Lepine & Sautter, Ann. de l'Institut Pasteur, 1938:61:519-526[11]
19	C	23	F	Nov.	1939	1500	+	+	Armstrong & Sweet, P.H.R., 1939, 54:673[7]
20	C	17	M	Nov.	1939	710	−	+	Armstrong & Sweet, P.H.R., 1939, 54:673[7]
21	W	13½	M	Jan.	1939	1330	−	+	Josephine B. Neal: *Personal communication*
22	W	16	F	Dec.	1939	800	+	+	Armstrong, Wallace & Ross, P.H.R. 1940, 55:1222[27]
23	W	26	M	Nov.	1939	2400	+	+	Braun, G. O.: *Personal communication*
24	W	21	F	Feb.	1940	1900	+		H. Saltonstall, Philadelphia: *Personal communication*
25	W	8	M	Sept.	.....	1400			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
26	W	16	M	Sept.	.....	330			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
27	W	33	M	Oct.	.....	63			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
28	W	20	F	Mar.	.....	409			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
29	W	33	M	Feb.	.....	304			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
30	C	15	M	May	.....	1800			Baird & Rivers, A.J.P.H., 1938, 28:47[36]
31	W	24	M	Sept.	1939	1600	+	+	Armstrong: *Unpublished case*[28]
32	W	32	M	Apr.	1939	500	+	+	Armstrong, Wallace & Ross, P.H.R. 1940, 55:1222[27]
33	C	24	M	Oct.	1938	970	+	−	Leichenger, Milzer & Lack, J. A.M.A., 1940: 436[41]

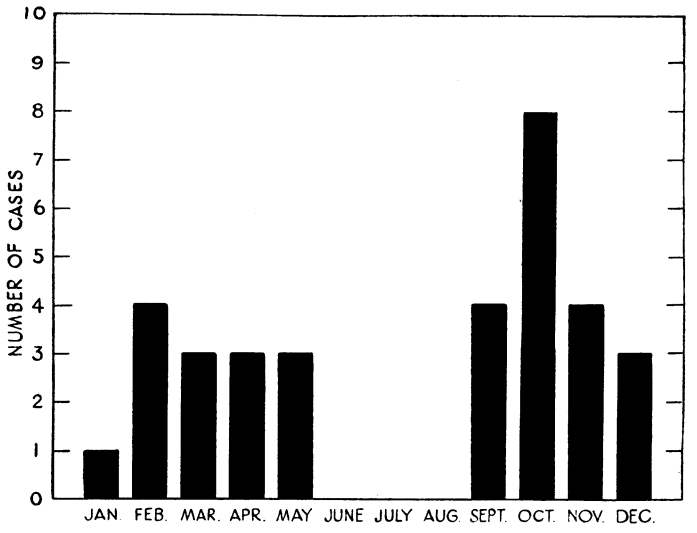

Fig. 2—Monthly distribution of cases of choriomeningitis virus infection.

mice convey the infection to only a portion of their normal cage-mates even after an exposure of one to two months (Kreis,[19] Haas[30]), Haas has, however, shown that congenitally infected mice are much more effective conveyors of infection and may transmit the infection to cage-mates after an exposure as short as one hour.

Moreover, the finding of 52 per cent of the mice trapped in homes harboring infected mice to be active carriers of the virus, in a study extending over several months, points to a persistent type of infection such as follows the congenital method of spread rather than toward an infection of mice from man.

Method of spread of infection between mice and man: The presence of the virus in the blood stream and the ready transmission of the systemic type of the infection by subcutaneous inoculations suggest the possibility of an insect transmission.

We have, moreover, succeeded in two instances out of several trials in transferring the infection by means of approximately 100 lice taken from an infected monkey and directly transferred to a normal one.

Attempts to transfer the infection by bedbugs, rat and mice fleas, and a bloodsucking mite have, however, uniformly failed. The virus could be detected in each species when the engorged arthropods were

emulsified and inoculated into normal mice within one hour of the infective feeding, but not after longer intervals. Attempts to convey the infection through biting were entirely negative.

Coggeshall[31] reported the transmission of choriomeningitis to one guinea pig through the bites of seven *Aedes aegypti* mosquitoes made five days after feeding upon an infected guinea pig. Shaughnessy and Milzer[32] succeeded also in infecting Rocky Mountain wood ticks by feeding them on infected animals and demonstrated that the infection could pass through the complete life cycle of the host. Controlled biting experiments were entirely negative but, when infected nymphs were put in the cage with normal guinea pigs, infection occurred. It is probable that these observations have little if any bearing upon the spread of choriomeningitis in nature, for established cases have been noted throughout the winter in latitudes where mosquitoes and ticks are scarce or absent during this period; while in June, July and August, the months when mosquitoes and ticks are prevalent in the northern hemisphere, there have been no proven cases reported (Fig. 2).

The seasonal distribution of cases and the respiratory symptoms so commonly noted in choriomeningitis infections, moreover, suggest a respiratory route of infection. The case of a laboratory worker reported by Lepine, who developed symptoms six days after splashing infectious material into her eye is, moreover, consistent with this route of infection as is the patient reported by Findlay[9] who developed the disease after cleaning a mouse infested shed.

The virus escapes from infected mice by way of the nasal secretions, urine, and feces, and it is conceivable that dust plays a part in the transfer of the infection to man, although it is possible that contaminated food may play a part; it appears, however, that experimental animals are more susceptible to freshly isolated strains given intranasally than they are by feeding. The investigations of Shaughnessy and Zichis[33] on guinea pigs suggest the possibility that the virus may also be able to pass through the unbroken skin.

Why are there not more human cases? In view of the extent of the mouse infestation and infection in the District of Columbia, it is natural to enquire why more human cases of choriomeningitis have not become apparent. As stated above, the recognition of the disease has been practically confined to the central nervous system types of the ailment, while the systemic type of the infection has probably escaped identification.

The fact that approximately 11 per cent of 2000 sera collected at random possessed neutralizing antibodies for choriomeningitis virus, while the donors largely deny a history of central nervous system disease at any time, points toward an unrecognized infection with the virus as being quite common.

It, therefore, seems probable that choriomeningitis virus infection is more common than is indicated by the occasionally occurring meningeal type of the infection. The reason more cases of this latter type do not occur is probably attributable to the efficient barrier which tends to protect the central nervous system against various infections.

Special considerations: The wide range of species susceptible to experimental infection with choriomeningitis along with the finding of naturally infected mice, monkeys, and dogs gives the virus an especial importance for those engaged in animal experimentation with other viruses. For instance, Dalldorf and Douglass[34] recovered choriomeningitis virus from four different dog spleens and found suggestive evidence of its presence in several others. Needless to state, the unexpected intrusion of this virus led to some temporary confusion in their canine distemper investigations.

A commercial manufacturer, likewise, has encountered choriomeningitis in the preparation of canine distemper tissue vaccine and Mollaret[35] has raised the question as to whether this virus may not have been responsible for some of the meningeal reactions observed in the early attempts to vaccinate against yellow fever by means of mouse tissue vaccine.

Diagnosis: Choriomeningitis should be considered whenever a lymphocytic type of meningitis of unknown etiology is encountered, whether or not it be associated with symptoms of encephalitis and encephalomyelitis. The case reported by MacCallum and Findlay[10] further shows that choriomeningitis may occasionally simulate poliomyelitis. These authors isolated choriomeningitis virus from the spinal fluid as well as repeatedly from the nasal secretions of this case. Furthermore, this infection must be considered in cases of sporadic "grippe-like" infections.

There are at present, however, no characteristic symptoms by which the clinician can with certainty diagnose this infection, although, the finding of a clear spinal fluid with a cell count above 1200, mainly lymphocytes, points, as Baird and Rivers[36] have stated and as our experience indicates, toward lymphocytic choriomeningitis. The diagnosis rests ul-

timately, however, upon either the isolation of the virus or the demonstration of a developing immunity.

The virus is most readily recovered from spinal fluid or blood drawn preferably at or prior to the height of the attack, and less regularly in later drawn samples. The virus has also been occasionally isolated from the patient's urine and nasal discharges. The inoculation of susceptible animals should be promptly carried out, or when delay is unavoidable the material should be promptly chilled and held near freezing.

A study of the serum for neutralizing or complement-fixing antibodies may also yield diagnostic evidence. Blood for such tests should be drawn with sterile precautions and handled without the addition of anti-coagulants or preservatives of any kind. This statement is prompted by the fact that we receive at the National Institute of Health many samples mailed from various parts of the United States which contain anti-coagulants, glycerine, or other preservatives. It is also surprising how frequently blood is mailed in cotton stoppered containers from which the serum, of course, uniformly escapes.

Virus-neutralizing antibodies are usually not demonstrable in the blood before 6 to 10 weeks following the onset of symptoms, but in our experience, they invariably appear in established cases. Howard,[37] however, reported two cases in which demonstrable virus-neutralizing antibodies failed to appear following the attack. Once established, neutralizing antibodies tend to persist for months or even years. Baird and Rivers[36] report the case of a child 8 years of age in which the antibodies disappeared after 8 months. We have, however, examined patients as much as 3½ years after the attack and found the antibodies undiminished.

It is desirable that blood be drawn early in suspected cases and again some 6 to 10 weeks following the attack. Both samples should then be tested for antibodies when a definite increase in the later drawn as compared to the earlier drawn specimen may be considered of diagnostic significance.

Complement fixation, as carried out by Lepine, Mollaret and Sautter[38] and by Smadel, Baird and Wall[39] is also a valuable diagnostic procedure. Complement fixing antibodies make their appearance earlier and tend to be less persistent than the slower developing neutralizing antibodies.

Treatment: There is no specific treatment of proven value known. The favorable experimental results secured in mice with prontosil by

Rosenthal, Wooley and Bauer[40] have not been consistent and when effective were apparently dependent upon early administration and more nearly approach prophylaxis than they do treatment.

Leichenger, Milzer and Lack[41] feel that sulfanilamide was effective in one patient observed by them in spite of the fact that the patient suffered four distinct relapses and the illness persisted for 4 months.

Spinal drainage is the one measure that frequently has afforded relief from the severe headache and vomiting. Cellular changes in the spinal fluid may persist for a considerable period following the disappearance of acute symptoms and accumulating instances where one or more relapses occur indicate that there is danger in discharging the patient to full activity before the spinal fluid has returned to normal.

Prevention: There are many details of the exact manner of spread for the virus yet to be determined; the findings reported do, however, suggest that prevention would be served by the construction of homes with a view to rendering them mouse proof and by reducing or eliminating mouse infestation from quarters frequented by people.

Poliomyelitis

Poliomyelitis is a disease that has been much studied but the investigations have, to date, singularly failed to answer definitely many fundamental epidemiological considerations relating to this disease, such as the method of spread of the virus, and the portal of its entry to the central nervous system. A noteworthy recently established fact is that the virus of poliomyelitis may exist in the intestinal tract of man from which it escapes, in readily detectable amounts, along with the dejecta. The significance of this fact is, however, a matter of controversy and any attempt to present the *pros* and *cons* of this discussion would be time-consuming and without hope of reaching a definite or convincing conclusion at this time. I shall, therefore, forego consideration of the controversy raised by this recent development and proceed to a consideration of the cotton rat and white mouse in poliomyelitis research.

In so far as availability, cost and such general considerations are concerned the cotton rat and white mouse are admirable laboratory animals. Their suitability for poliomyelitis research is, however, dependent upon the degree and uniformity of susceptibility, the incubation period, and the definiteness of the symptoms. It is these factors which I wish to consider.

Susceptibility to new strains: The susceptibility of the cotton rats to new strains is apparently low; for instance, in 1937 and again in 1938 we were able to transmit a monkey-adapted strain of poliomyelitis from Lansing, Michigan, to one of two and one of eleven cotton rats, respectively, after an incubation period of 29 days in each instance, but attempts at further passage failed. In 1939 one of four inoculated rats developed symptoms and from this rodent the strain has been successfully carried through fifty-five successive transfers and after seven generations was successfully conveyed to white mice.

During 1939 we had one of four cotton rats develop symptoms following inoculation with a strain of virus from Niagara Falls after an incubation period of 41 days. We also produced symptoms in one of several rodents with a strain of virus from Detroit and succeeded in carrying it through three generations. Further attempts at transfer failed in both instances, as did attempts to convey six additional strains to cotton rats.

Other investigators have attempted to convey many strains of poliomyelitis to cotton rats but with few successes. For instance, Jungeblut and Sanders[42] succeeded, three times, in passing a virus to cotton rats, employing the monkey-adapted S.K. poliomyelitis strain, which was originally of fecal origin. After passage in cotton rats the virus was found to be pathogenic for white mice, in which species it showed extreme potency and a remarkable capacity to pass the central nervous system barrier, but with mouse passage it tended to lose its pathogenicity for monkeys. This virus differs markedly from the mouse-adapted Lansing strain of virus, which was of nervous system origin, and it is conceivable that neuro and fecal strains may behave differently. The authors kindly supplied me with their strain of virus and I regret that I have not been able to work with it, due to the stress of more urgent but not necessarily more important duties. Therefore, I am not able to express an authoritative opinion on the authors' contention that their murine virus is immunologically one of poliomyelitis. The most convincing evidence set forth by the authors in support of this contention is, perhaps, the fact that the pseudoglobulin concentrate from an R.M.V. antipoliomyelitis horse serum "neutralized" their murine virus in high concentrations. It is noted, however, that an R.M.V. antipoliomyelitis serum from a convalescent monkey gave no neutralization against the same strain of virus. The authors explain this discrepancy in behavior of the two anti-R.M.V.

sera as due to "The enormous potency of the hyperimmune horse serum breaking down immunological strain differences." The authors, however, also mention in passing that diphtheria antitoxin (pseudoglobulin) in several tests also inactivated large doses of murine virus.

It is difficult to harmonize these results on the basis of specific neutralization and I am wondering if the authors have considered the possibility that their concentrated sera may have contained a preservative. I make this inquiry because a preservative is required, by Federal regulation, to be added to serum concentrates if prepared for human use and intended for inter-State sale. Moreover, many producers follow Federal requirements in preparing sera for intra-State use. The presence of a preservative such as merthiolate or phenol, acting for 1½ hours at 37° C. and over night in the ice box prior to inoculation, would have opportunity to destroy a portion of the virus; the result would simulate a true neutralization and supply a ready explanation for the odd results secured by the authors. Should this possible explanation be the correct one, the remaining evidence of immunological relationship with poliomyelitis would be so slight as to cause one to doubt whether the murine virus was related to poliomyelitis virus at all.

A personal communication has recently been received from another investigator who claims to have transferred the P.M.V. strain of poliomyelitis to cotton rats. He kindly sent me some of this virus strain and when inoculated into cotton rats it produced symptoms similar to those caused by the Lansing strain, but it has not been further identified at the National Institute of Health.

The evidence thus indicates that the cotton rat is a far less satisfactory animal for the primary isolation of poliomyelitis strains than is the monkey.

Susceptibility to Lansing strain: Cotton rats and white mice appear to be quite susceptible to the adapted Lansing strain. The incubation period in the former is usually 3 to 8 days, while in white mice symptoms become apparent usually in 2 to 10 days following inoculation, but the interval has been observed to be as short as 24 hours and as long as 93 days. In both these rodent species the symptoms are very distinct, paralysis of one or more legs being usually first observed, while paralysis of the neck, back, and tongue have been seen. Death occasioned by respiratory paralysis usually takes place within 12 to 48 hours of the first recognized symptoms. White mice almost uniformly succumb when .03 cc.

of a suspension of infected mouse brain in a dilution up to 1:500 is employed and a portion of the inoculated mice will develop symptoms with dilutions as high as 1:10,000. Occasionally an animal recovers after having developed symptoms. In such instances of recovery muscle atrophy and deformities usually develop.

Route of inoculation: The only successful route of inoculation for rodents employing the Lansing strain of virus consists in the direct inoculation of the central nervous system. Intranasal, intraocular, subcutaneous, intraperitoneal inoculations, as well as feeding, have all failed to induce infection. Unless these negative results can be overcome it is difficult to see how these rodents can contribute much toward elucidating the natural route of infection or transit of the virus in man.

Immunity: Mice and cotton rats which have recovered from paralytic attacks induced by the Lansing strain are solidly immune to intracerebral inoculation with the virus.

Humoral immunity: When Lansing virus-infected mouse brains are inoculated under the skin of mice or cotton rats they develop potent humoral antibodies readily demonstrable by the serum-virus protection test in mice or cotton rats. Such antibodies exert a slight protective action against intracerebrally inoculated minimal infective doses of virus (Table II). It is conceivable, however, that the protective action may be due to the artificial method of inoculation since the needle in some instances probably produces some bleeding at the inoculation, where the plasma coming into direct contact with the virus would, if immune, tend to prevent infection. The artificial method of inoculation necessary to bring about infection in mice, therefore, tends to cast doubt upon the significance of the degree of protection afforded subcutaneously immunized mice when minimal infective doses of virus are employed.

Humoral immunity in the population: Approximately 300 human sera from various sources have to date been submitted to the serum-virus neutralization test in white mice, employing the mouse-adapted Lansing strain of virus. The test is rapid, requires but .45 cc. of serum, and the results are usually definite, easily read, and readily repeatable (Table III).

This group of 300 tested human sera is larger than any series yet reported by a single laboratory employing monkeys; however, we consider that we have just begun. No detailed analysis of our results will, therefore, be attempted at this time. In general, the results secured to

TABLE II

INFLUENCE OF SUBCUTANEOUS INOCULATION UPON LATER INTRACEREBRAL INOCULATION

1940. *Experiment 1109.* *Immune Status of Mice*	*Virus subcutaneously 7-12-40*		*Virus dilution intracerebrally .03cc.*	*No. mice intracerebrally inoculated*	*Deaths by Days Following Inoculation*																					*No. mice surviving*	*Per cent of mice dying*
Immune Status of Mice	6/27	7/3			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Immunized	+	+	1/500	30	*3			10	2		1		2						1	3		3				5	81.5
Controls	−	−	1/500	35	*2	2		13	1			1	2		3	1	4	2	1							3	90.9
Immunized	+	+	1/2500	30		1	1	3		2					1	2	1								1	18	40.0
Controls	−	−	1/2500	33	*1			8	1			1			3		2			1		2	1			13	59.4

*Indicates deaths: Excluded as due to Trauma. Immunized and Control Mice bled July 11, 1940.

TABLE III.

SERUM-VIRUS PROTECTION TEST WITH WHITE MICE

PROTOCOL

*Serum	Virus Dilution inoculating 4 mice	Deaths by Days Following Serum-Virus Inoculation																				Survived	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
P-131	1/10																						4	
	1/20																						4	Immune
	1/40																						4	
P-132	1/10						1	1			1			1									0	
	1/20							2						2									0	Not
	1/40							1		1													2	Immune
P-133	1/10			1						1				2									0	
Negative	1/20		1					1				1					1						0	Not
Control	1/40							1		1			1							1			0	Immune
P-134	1/10																						4	
	1/20																					1	3	Immune
	1/40																						4	
P-135	1/10																						4	
	1/20																						4	Immune
	1/40																						4	
P-136	1/10																						4	
Lennett +	1/20																						4	Immune
Control	1/40																						4	
P-137	1/10							2	1	1													0	Not
	1/20					1				1	1												1	Immune
	1/40										1	1			1			1					0	

* .1cc. undulated + .15cc. various dilutions of virus held for 1 hour at room temperature. .03cc. of mixture inoculated intracerebrally.

date are in agreement with those secured in monkeys. For instance, 65 per cent of the sera possessed definite immunity, 6.5 per cent partial immunity, and 28.5 per cent no immunity; the percentage of immune sera increased with age of the donors, and children living in institutions tended to show a higher proportion with immunity than was the case with the same age group living in urban dwellings.

The test is so inexpensive and can be so rapidly performed that it is now possible to study adequately humoral immunity in groups and geographical units of the population and by repeated tests to follow immunity development in individuals; and in this way to elucidate, it is hoped, the epidemiology of the disease, at least, in so far as one strain of virus is concerned.

Chemotherapeutic studies: Cotton rats and white mice along with the Lansing strain of virus are also being utilized in the search for curative agents in poliomyelitis and they will permit studies to an extent practically impossible with the monkey.

Is the Lansing strain exceptional or peculiar? In view of the various studies now being pursued with the Lansing strain of virus it seems appropriate to inquire if this strain of virus is exceptional or peculiar. The virus originated from what was clinically considered to be a typical case of fatal bulbar poliomyelitis. The clinical and pathological picture produced in monkeys with this strain of virus both before and after its transmission to cotton rats and white mice is identical and confirms the opinion that the Lansing strain is one of typical poliomyelitis. The finding of virus-neutralizing antibodies in 71.5 per cent of approximately 300 human sera from various localities of the United States further indicates that the Lansing strain of virus is immunologically a commonly occurring one. The finding that four out of seven antisera collected from monkeys convalescent from various strains of poliomyelitis protected mice against the Lansing strain of poliomyelitis virus points in the same direction.

The sera tested which gave positive results were as follows:

		Results
1.	From Lennette (P.M.V.)	+
2.	From Niagara Falls, 1938	+
*3.	From S. D. Kramer, Michigan (Rhesus 91) . .	+
*4.	From S. D. Kramer, Michigan (Rhesus 83) . .	+
5.	From Aycock, Boston	−
6.	From Charleston, S. C., 1939	−
†7.	From S. D. Kramer, Michigan (Rhesus 41) . .	−

The failure of three convalescent monkey sera to show protection against the Lansing strain appears to be in agreement with strain difference indicated by the work of Burnet and Macnamara,[43] Weyer,[44] Paul and Trask,[45] Kessel and co-workers,[46] and others. This apparent confirmation, however, rests on an assumption since we have not demonstrated that the three sera, which lacked apparent neutralizing antibodies for the Lansing strain, actually possessed antibodies at all, since the homologous viruses had not been adapted to mice and we did not feel justified in sacrificing monkeys for the purpose.

* A communication from S. D. Kramer subsequent to delivery of this paper reveals that sera 3 and 4 were from monkeys immunized against the Lansing strain of virus.
† Animal number 7 died of tuberculosis.

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